We now demonstrate, to the best of our knowledge for the first time by direct observation, that anaerobically drawn arterial blood of sickle patients shows birefringence in many red cells and therefore RAPs exist, which we confirm by EM observation of aligned polymers. RAPs exist not only under hypoxemic conditions, when they can be explained by limited solubility due to the presence of deoxyHbS, but also when hypoxemia is absent. RAPs without hypoxemia imply that slow depolymerization kinetics are responsible. One minute of voluntary hyperventilation and (separately) brief nasal oxygen greatly decrease RAPs. RAPs increase during sleep. We attribute these results to accelerated depolymerization at increasing levels of oxygen that cooperatively induce polymer fracture (fracture, using CO, exhibits a 4.7 power dependence on pCO). These results and the interdependent progress of oxygen saturation, partial pressure, fracture rate and remaining polymer that we model bear on pathogenesis and particularly on vaso-occlusive crises, which result from red cell rigidification and from cellular adhesion due to polymer-dependent cellular damage. Under these mechanisms, the lungs may play an important role in initiating pathology; and remediation of dysfunction by breathing assists is potentially prophylactic.

2873-Pos Board B28

Sickle Cell Therapy and the Kinetics of Polymerization in the Presence of Ligands

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Sickle cell disease results from a mutation of normal human hemoglobin that renders it capable of polymerization once oxygen is delivered. The process of deoxygenation involves quaternary as well as tertiary changes in Hb, and both changes appear to be involved in permitting HbS to assemble into the stiff polymers that distort cells and lead to the occlusion of the microvasculature. Kinetics are central to the disease, because if polymerization is slow enough to occur in the veinous return, the polymerization can be reversed upon reoxygention with minimal if any consequences. We have recently completed a detailed study of the polymerization kinetics of HbS in the presence of ligands. We have found that the kinetics are consistent with equilibrium measurements that show singly ligated HbS molecules will polymerize with only about 0.35 the probability of a deoxy HbS molecule. Given knowledge of these highly concentration sensitive rates, and a distribution of intracellular concentrations, we can calculate the likelihood of sickling at various points in the microcirculation. Because of the finite rate of oxygen delivery, we find sickling is most likely at the exit of the capillaries, which is where obstruction has been observed with intravital microscopy. When this is combined with the results for mixtures of fetal hemoglobin (HbF) or HbA, it becomes possible to determine therapeutic targets for the reduction of rates of sickling.

2874-Pos Board B29

Biophysical Studies Evaluating the Potential Physiological Relevance of the Hemoglobin Associated Nitrite Anhydrase Reaction as a Pathway to Generate S-Nitrosothiols from Low Levels of NO and Nitrite

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Hemoglobin (Hb) has been implicated in nitrite-mediated mechanisms that generate bioactive forms of nitric oxide by the reaction of deoxy Hb with nitrite to produce NO (Nitrite Reductase) is viewed as part of the mechanism since the generated NO is readily scavenged, raising questions as to how free NO could escape from the red blood cell. A proposed nitrite anhydrase reaction (NA) between met-Hb and both nitrite and NO to yield N_2O_3, a potent S-nitrosating agent capable of generating longer lived S-nitrosothiols, could address this limitation. Concerns regarding the physiological relevance of the NA reaction stem from the low affinity binding of nitrite to met-Hb and the competition of reductive nitrosylation which generates NOHb. We have identified a relatively stable spectroscopically distinct species generated from met-Hb and the combination of NO and nitrite that can S-nitrosate glutathione. We have tentatively assigned this species to the purported NA intermediate in which N_2O_3 is bound to the heme. The intermediate can be efficiently generated under conditions of low NO and low nitrite. We find that when NO binds to met- Hb, the affinity for the subsequent binding/reaction of nitrite dramatically increases. using sol-gel matrices to trap R and T forms of Hb, we find that for the T state the reductive nitrosylation pathway is favored, whereas for the R state the NA pathway is favored implying a control mechanism for the production of S-nitrosothiols via the NA pathway. Similar studies using HbE, a mutant Hb having an enhanced redox potential, support a mechanism whereby the R/T dependent redox potential is the primary factor that controls the partitioning between the RN and NA reactions of Hb.

2875-Pos Board B30

Modulation of Nitric Oxide Reactivity by Heme Posttranslational Modification in the Cyanobacterial Hemoglobin, GlbN

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Synechococcus sp. PCC 7002 is a model cyanobacterium capable of thriving under conditions that promote the buildup of reactive oxygen and nitrogen species (ROS/RNS). This organism harbors a hemoglobin (GlbN) that is thought to aid in the detoxification of RNS including NO.

GlbN achieves hexacoordinate heme (FeIII/FeII) binding using His70 (proximal) and His46 (distal). In vitro, this coordination scheme protects against H2O2-induced damage, facilitates electron transfer (ET), and lowers redox potential. Under reducing conditions, His117 attacks the 2-vinyl group to form a covalent crosslink. The irreversible posttranslational modification (PTM) of GlbN yields GlbN-A. Ligands such as CO, O2, and NO inhibit the facile PTM. This and other observations suggest that both GlbN and GlbN-A are active in the cell. How does the His-heme PTM influence GlbN reactivity towards NO?

NMR and optical spectroscopies were used to study the differences in NO binding, NO oxidation, ET, and NO reduction. We observed that GlbN and GlbN-A can form unusually stable diamagnetic FeIII-NO complexes. Both FeII-O2 proteins appear capable of NO dioxygenation, where ET is typically rate-determining. Each GlbN exhibits facile ET, with measured self-exchange rates slightly slower than cytochrome b5. A difference in NO reactivity is observed under strongly reducing conditions: surprisingly, unmodified GlbN is capable of reducing NO to nitrosyl hydride (HNO). Additionally, FeII-NO binding in GlbN results in immediate heme loss.

The results provide some insight into the ability of GlbN to protect the cyanobacterium from RNS/ROS. The data suggest that GlbNs can serve as NO dioxygenases, but may not require a dedicated reductase because of their propensity for facile ET. Additionally, an unusual NO reductase-like activity may also exist for GlbN, and the His-heme PTM appears to eliminate this chemistry.

Supported by NSF MCB 0843439

2876-Pos Board B31

Redox-Controlled Proton Gating in Bovine Cytochrome *C* Oxidase

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Cytochrome *c* oxidase is the terminal enzyme in the electron transfer chain of essentially all organisms that utilize oxygen to generate energy. It reduces oxygen to water and harnesses the energy to pump protons across the mitochondrial membrane in eukaryotes and the plasma membrane in prokaryotes. The mechanism by which the oxygen reduction reaction is coupled to proton pumping remains unresolved, owing to the difficulty of visualizing proton movement within the massive membrane-associated protein matrix. Here, with a novel hydrogen/deuterium exchange resonance Raman spectroscopy method (1), we have identified two critical elements of the proton pump: a proton loading site near the propionate A group of heme *a*, which is capable of transiently storing protons uploaded from the negative-side of the membrane prior to their release into the positive-side of the membrane and a conformational gate that controls proton translocation in response to the change in the redox state of heme *a*. These findings form the basis for a new molecular model describing the mechanism, by which unidirectional proton translocation is coupled to electron transfer from heme *a* to heme a_3 associated with oxygen chemistry occurring in the heme a_3 site during enzymatic turnover.

2877-Pos Board B32

Reduction of Iron Center Enhances the Mechanical Stability of Cytochrome B562

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Because redox energy can be converted into conformational energy, heme redox proteins offer a unique opportunity to examine the coupling between redox reactions and protein mechanics. Here, we use Atomic Force Microscopy-based single-molecule force spectroscopy (SMFS) to directly examine the effect of heme and its oxidation state on the mechanical properties of cytochrome b562 (cyt b562). We found that cyt b562 is mechanically stronger in its reduced state as compared to its oxidized state. In addition, we discovered the shortening of